Bioleaching of Metals (Cu, Au and Ag) From Waste Computer Printed Circuit Boards Using Cyanogenic Microorganisms

SYNOPSIS

Submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Biotechnology

By

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Enrolment No. 136566

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WAKNAGHAT-173234, HP, INDIA

February, 2018

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1. INTRODUCTION

Over the past decade, an exponential increase in electronic waste (e-waste) generation caused by the high consumption of electronic devices and their early obsolescence has been observed [1]. Globally, 44.7 million metric tons (Mt) of e-waste was generated in 2016 and is projected to increase up to 52.2 Mt by 2021 [2]. The rapid development of the electronic industry has impacted the environment in two ways: a) the 4–5% increase in e-waste generation per year has resulted in large volumes of e-waste being disposed off and landfilled and b) the amount of mining for precious and rare earth metals from primary ores has increased [3, 4]. Balde et al. [2], estimated approximately 2,164, 1.6 and 0.5 Kiloton (Kt) of Cu, Ag and Au, respectively, in the e-waste produced in 2016. E-waste has been treated as a "secondary ore" in "urban mining" due to the presence of precious metals [e.g., Au, Ag, palladium (Pd)] [5]. The concentration of precious metals in the e-waste of printed circuit boards (PCBs) is higher than that in primary ores; for example, the ore deposit of both Ag and Au is <10 g/ton compared to the PCB deposits of Ag and Au at 1,000 g/ton and 250 g/ton, respectively [6]. Thus, there is a need to establish proper management system to recover precious metals from e-waste. The interest in e-waste recycling has risen, especially with the aim of recovering precious metals from waste PCBs.

The sheer volume of e-waste, scarcity of natural resources, and presence of precious and rare earth metals have emphasized the need of appropriate e-waste management strategies. The global e-waste management market was expected to grow at Compound Annual Growth Rate (CAGR) of 23.5% during 2014-2020. Thus recovery of Cu, Au and Ag from e-waste (secondary resource) will not only lead business opportunities in this sector, but also reduces the load on natural mines. The increased demand, high cost of extraction, shortage of high quality ores and increased prices have attracted miners towards formal e-waste recycling and management [7].

Several hydrometallurgical and pyrometallurgical methods of metal recovery are currently practiced [3, 7]. However, considerable quantities of secondary byproducts are generated, which is a limitation of these methods. For example, pyrometallurgical treatment generates large amount of slag, fumes of heavy metals (metals with low melting point like mercury, lead and cadmium), and may lead to formation of mixed halogenated dioxins and furans (especially, if the scrap contains plastic with brominated flame retardants (BFRs)) [8]. Whereas, hydrometallurgical methods generates spent acid, toxic gases, large amount of sludge and liquid waste [8, 9]. In contrast, bioleaching is an environmentally friendly and cost-effective technology that has been employed by various researchers to recover metals [10-12]. Bioleaching lowers the demand for resources such as energy, ores, and landfill space, but its application is still in its infancy [7]. A variety of chemolithotrphic bacteria (e.g., *Acidithiobacillus thiooxidans*, *Acidithiobacillus*

ferrooxidans) cyanogenic bacteria (e.g., *Chromobacterium violaceum*, *Pseudomonas* sp., and *Bacillus megaterium*) and fungi (e.g., *Aspergillus niger*, *Penicillium simplicissimum*) are known for their ability to mobilize metals from e-waste [10, 12, 13]. Cyanogenic bacteria such as *Chromobacterium violaceum*, *Pseudomonas* sp., and *Bacillus megaterium* produces hydrogen cyanide (as a secondary metabolite) and form water soluble metal cyanide complexes under alkaline conditions (pH 8-10) [14, 15]. As the pKa of HCN is 9.3, conducting metals dissolution under alkaline conditions enhances the availability of cyanide ions for bioleaching. Cyanide production by these microorganisms occurs at early stationary phase, forming the non-dissociated form of hydrocyanic acid (HCN) and cyanide ions (CN-). At physiological pH (7), cyanide is mainly present as hydrogen cyanide [16]. However, bacterial growth under alkaline conditions is a challenge in such processes.

It has been reported that higher concentrations of e-waste are toxic to bacteria [16, 17], and metal bioleaching is strongly dependent on the metabolic activity and status of the cell [7]. In this context, indigenous microorganisms are favorable over foreign microorganisms, as indigenous microbes have the physiological and metabolic machinery necessary to resist the contaminant due to natural selection [18]. Mousavi et al. [19], reported higher Zn extraction (87%) from low-grade sphalerite concentrate using native strain *Sulfobacillus thermosulfidooxidans* compared to laboratory reference strains or control conditions. Romo et al. [20], showed 70% of copper (Cu) removal from low grade ore using a native consortia (*At. ferrooxidans* and *At. thiooxidans*), showing significant difference with other consortia which removed only 35% of Cu, during the same time period of 35 days. Keeping all these factors in view the present work was carried out with an aim to explore native microbial communities capable of bioleaching of Cu, Au and Ag from e-waste, their toxicity assessment and dose-response analysis and optimization of process parameters to enhance the metals extraction. The present study was first to 1) report on isolation of bacterial strains from e-waste recycling facility (Panipat, Haryana) and abandoned gold mine (Solan, Himachal Pradesh) with an aim to exploit them for bioleaching of metals from discarded CPCBs and 2) dose-response assessment of e-waste toxicity upon bacterial strains.

2. OBJECTIVES:

I. To determine the heterogeneity in the metals content of waste computer printed circuit boards (CPCBs).
II. Isolation, screening and characterization of potent bacterial strains capable of bioleaching of metals (Cu, Au, and Ag) from waste CPCBs.
III. E-waste Toxicity assessment and dose-response analysis of potent bacterial strains.
IV. Optimization of process for maximum recovery of metals (Cu, Au and Ag) from waste CPCBs.

3. MATERIALS AND METHODS

3.1 Determination of the heterogeneity in the metals content of waste CPCBs

Five different samples of waste computer printed circuit boards (CPCBs) were procured in zipper storage bags from the storehouse of Exigo Recycling Pvt. Ltd., Panipat, Haryana, India. The particle size of waste CPCBs was determined using standard sieves (Standard test sieves as per IS 460: 1962). Recyclers collect the discarded electronic equipment's from various parts of India and take out PCBs from the obsolete gadgets. In the industry, the discarded gadgets undergo various physical and mechanical separation processes like segregation and manual dismantling (Fig. 1). The metallic content of waste PCBs was determined using acid digestion with aqua regia (HNO_3: HCl = 1:3), a protocol used in several studies [7, 12, 16]. The dissolved metals ions were quantified using atomic absorption spectrophotometry (AAS) (AAnalyst 400, PerkinElmer).

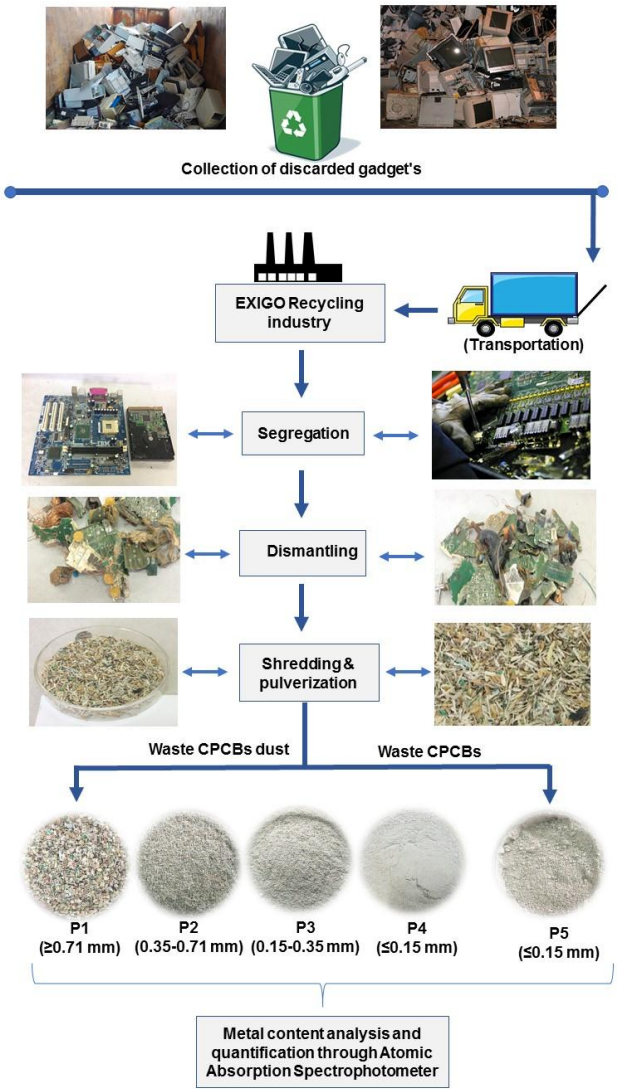

Figure 1: Schematic diagram representing e-waste processing at EXIGO recycling industry, Panipat, Haryana (ISO4001 & OHSAS 18001 Certified).

3.2 Isolation, screening and characterization of potent bacterial strains capable of bioleaching of metals (Cu, Au, and Ag) from waste CPCBs.

Soil **s**amples and e-waste refuse of industry were collected from abandoned gold mine of Solan, Himachal Pradesh and e-waste recycling facility Panipat, Haryana, India. The samples were stored at -20°C until analysis. The microbial community of gold mine soil (Solan, HP, India) and e-waste refuse of recycling facility (Panipat, Haryana) were enriched in LB medium (Luria broth) (100 mL in 250 mL Erlenmeyer flask) containing sterilized waste CPCBs at respective concentration. One gram of representative sample from both sites was inoculated in separate LB medium flasks supplemented with 10 g/L pulp density of e-waste (particle size ≤150 µm). The experimental flasks were incubated at 30ºC in an incubator shaker (Thermo Scientific MaxQ 8000) at 150 rpm for 6 days. After incubation of 6 days, 5 mL (10^8 CFU/mL) of inoculum was transferred sequentially to the 2^{nd} set of flasks containing fresh medium supplemented with 25 g/L pulp density of waste CPCBs. The sequential enrichment was continued over a period of 30 days at an increasing e-waste pulp density of 50, 75 and 100 g/L, respectively. After 30 days, samples from the flask containing 100 g/L pulp density were serially diluted and spread plated on nutrient agar plates. The plates were incubated at 30ºC for 2-4 days and observed for the appearance of colonies after every 24 h. Morphologically distinct colonies were selected and repeatedly sub-cultured to ensure the purity of the bacterial cultures . An un-inoculated control was incubated in parallel. The isolated bacterial strains were screened for their metal solubilization potential from waste CPCBs. In order to screen bacterial isolates for bioleaching of precious metals (Cu, Au and Ag); two-step bioleaching process was used as per protocol given by Pradhan and Kumar [12].

The identification of bacterial strains was done by 16S rRNA sequencing. Bacterial DNA was extracted by using Wizard genomic DNA purification kit (Promega) as per manufacturer instructions. The Polymerase Chain reaction (PCR) amplification of 16S rRNA gene was done by using universal primers 27F and 1492R. The PCR reaction of 20 µl volume contained 50 ng of template genomic DNA, 5 pmole of forward and reverse primers and PCR master mix (promega) as per protocol given by Kumar et al. [7]. The amplified PCR product (1465 bp) was separated on 1.5% agarose gel electrophoresis and then sequenced (Xcelris Labs Limited, India) using Sanger sequencing method. The obtained sequences were analyzed and submitted to NCBI GenBank database.

3.2.1 Cyanide Analysis: Cyanide production by bacterial isolates were estimated at their early stationary phase. The bacterial cells were grown in LB medium supplemented with 5 g/L glycine and incubated for 48 h. After 48 h, the culture was centrifuged at 7000 rpm for 10 minutes to remove the biomass. The supernatant was collected and stored in refrigerator at 4°C. The samples were then sent to Jeedimetla Effluent Treatment Ltd., (JETL) Hyderabad, India. The cyanide production was tested at JETL as per USEPA method 4500 CN^-/E.

3.3 E-waste Toxicity assessment and dose-response analysis of potent bacterial strains

To prevent failure of metal bioleaching; e-waste toxicity assessment and dose-response analysis of potent bacterial strains is of great importance [18]. On the basis of bioleaching for Cu, Au an Ag; four potent bacterial strains and reference strain *Chromobacterium violaceum* (MTCC-2656) were investigated for their e-waste toxicity tolerance. The bacterial cells of each strain were exposed to different e-waste pulp densities viz. 10, 50, 100, 150, 200, 300, 400 and 500 g/L. The plate count method was adopted to determine the toxicity tolerance. The flasks were inoculated with 1% (*v/v*) of inoculum containing 1.4×10^5 CFU/mL and sterile e-waste at respective pulp densities [21]. A separate control which contained bacterial cells without e-waste was run in parallel. All the flasks (in duplicates sets) were incubated at 30°C and 150 rpm for a time period of 24 h followed by enumeration of bacteria on nutrient agar medium. The bacterial growth was measured in terms of colony forming units (CFU)/gram of e-waste pulp density.

Dose-response is a quantitative analysis which provides estimates about the maximum treatment concentration and 50% effective concentration (EC_{50}) allowable for metals bioleaching [18]. Therefore, data generated from the toxicity assessment studies were plotted to generate dose-response curve using the GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, California). The percent inhibition response was computed as per equation (1):

$$\% \text{ IR} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \qquad (1)$$

Where *IR*- Inhibition Response
Control- bacterial growth in the absence of e-waste
Test- bacterial growth in the presence of e-waste

The %IR data obtained at various e-waste pulp densities was subjected for nonlinear regression. The dose-response curve was determined using log (agonist) versus normalized response-variable slope procedure in GraphPad Prism 6 [22].

3.4 Optimization of process for maximum recovery of metals (Cu, Au and Ag) from waste CPCBs

3.4.1 Two-step bioleaching: In the bioleaching process, bacterial cells were inoculated with an inoculum size of 5.0 mL (approximately 10^8 CFU/mL) followed by incubation at 30°C and 150 rpm for 48 h. After 48 h of incubation, sterilized (121°C, 15 psi and 30 min) waste CPCBs (10 g/L) of particle size ≤150 µm were added to the experimental flasks aseptically. The flasks were again incubated under same set of conditions for additional time period of 7 days. An appropriate control containing medium and sterilized waste CPCBs was run in parallel. After 7 days, media in flasks were filtered and centrifuged (Eppendorf, 5804 R) at 7000 rpm for 10 minutes to remove the bacterial cells and residual WPCBs from the medium. The supernatant obtained was further filtered through 0.45 µm filters to warrant particle free suspension and analyzed for metal ions using AAS (AAnalyst 400).

3.4.2 Optimization of experimental parameters using one-factor-at-a-time-approach: The bioleaching of metals from waste CPCBs using cyanogenic microorganisms is influenced by pH [14, 16], pulp density [23], temperature, and glycine concentration (precursor) [11, 15]. Therefore, the aforementioned factors were tested on variable ranges of pulp density (10, 50, and 100 g/L), glycine concentration (2.5, 5.0, 7.5, and 10.0 g/L), temperature (25, 30, 35, and 40°C), and initial pH (7, 8, and 9) for maximum metal mobilization from waste PCBs in the present study.

3.4.3 Optimization using response surface methodology (RSM): RSM is a group of mathematical/statistical techniques, which is used to study a functional relationship/interaction between responses of interest and several variables which affect the response [24]. Therefore, in our study, four factors including pulp density, initial pH, temperature, and additive (glycine) concentration, were optimized to enhance the recovery of Cu, Au and Ag by central composite design of response surface methodology (CCD-RSM). The total number of experimental runs in CCD were equal to $2^k + 2K + n_0$ in which 2^k is the factorial run, $2K$ is the axial run, n_0 is number of central point replications, and K is number of independent variables [25].

A total of 30 experimental runs were predicted by Design-Expert Software Version 10 (Stat-Ease, Inc., Minneapolis, Minnesota, USA). All the experimental runs were performed in duplicates. To determine the interactions among the variables, data obtained from the experimental runs was modelled. The significance of interaction between variables and responses was determined by ANOVA using Design-Expert Software Version 10. Further, additional experiments were performed on the optimal conditions predicted by software to validate the predicted results of the mathematical model with experimental results.

3.4.4 Bioleaching of metals enriched waste P5

a) Pretreatment of waste CPCBs-P5 using nitric acid: To remove the interference of base metals especially copper during bioleaching of gold; nitric acid pretreatment was performed. The 10 g of CPCBs waste was added to 30 mL of nitric acid (6 M) at room temperature under shaking conditions for 2 h. After 2 h of shaking, the mixture was centrifuged at 5000 rpm for 10 minutes and 5 mL of the sample was taken for metals content analysis. The residual waste was washed with deionized water and the cycle was repeated until traces of blue-green colour disappears. The pretreated waste CPCBs were dried, weighed and subjected to metal content analysis using aqua regia (HCl: HNO_3 = 3:1) [9, 16].

b) Comparative bioleaching study of pretreated and untreated waste: The bioleaching capabilities of bacterial strains against pretreated and untreated waste were tested under previously optimized condition using two-step bioleaching process. Out of five bacterial strains, only three bacterial strain were used based on their metals mobilization potential.

3.4.5 Characterization of the residual e-waste: Owing to its hazardous nature, residual waste CPCBs after metals recovery were characterized for it metals content analysis before disposal using aqua-regia based digestion method.

4. RESULTS AND DISCUSSION

4.1 Determination of heterogeneity in the metals content of waste CPCBs

The metal content of P4 and P5 of same particle size ≤0.15 mm were analyzed and results were presented in Table 1. Among all the metals analyzed Cu and Fe were dominated in both P4 and P5, respectively. This may be due to use of high amount of Cu for plating, coating, and layering in the circuitry of CPCBs. Whereas Fe is used for magnets and metal alloys (e.g. Fe–Si) in the printed circuit boards [26]. The amount of Cu in P4 and P5 was 2.34% and 7%, respectively. Precious metals like Au and Ag in P4 and P5 were significantly lower compared to base metals. The Au content in P4 and P5 was 0.008% and 0.03%, whereas Ag content was 0.04% and 0.14%, respectively. From the literature review, variation in the metallic content of waste CPCBs was also observed; for example, the Cu content reported by different researchers ranged from 2.34% to 39.2%, whereas Au content was ranged from 0.001 to 0.11%, respectively [27-29]. Other metals such as Ni, Co, Cr, and Zn were also present in significant quantities in both wastes i.e. P4 and P5. The variation in the metallic content of CPCBs is attributed to source, type of e-waste used and industrial pre-treatment processes like segregation, manual dismantling, shredding and pulverization [12, 30]. Additionally, the overall metallic content was higher in P4 and P5. The reason behind low concentration of P4 is; the waste CPCBs used was the dust generated during physio-mechanical processes at industry, whereas the P5 used in present study was a virgin waste.

It was observed that metals content varied with respect to different particle sizes. The highest concentration of Cu was found in the CPCBs of larger particle size (≥0.71 mm). Further, it was observed that as the particle size decreased from ≥0.71 mm (29.29%) to ≤0.15 mm (2.34%), the Cu content also decreased. Similar results were reported by Sun et al. [26]; where, Cu content increased with increase in particle size from <0.2mm (16.04%) to >1mm (38.34%). This may correspond to high ductility of Cu, which distributes it more in larger size fractions. The amount of precious metals like Au (0.008%) and Ag (0.04%) were high in case of small particle size fraction i.e. ≤0.15 mm. The low recovery of precious metals (Au & Ag) in the larger particles size fractions may be because of low participation of precious metals and high amount of Cu which, might have hindered the recovery of metals. Further, the precious metals in waste CPCBs are in a low quantity which are further entrapped within the metals present in bulk quantities and less exposed to lixiviant in larger size fractions. However, size reduction may have liberated the entrapped precious metals, increased their participation and contact surface for lixiviant. Hence, high concentration of Au and Ag obtained in lower size fractions. The variation in the metals content of waste CPCBs showed a large heterogeneity for example, the amount of Cu varied from ~2.34% to ~29.29% in the waste CPCBs of different size fractions. The heterogeneity in the metal content of waste CPCBs of the same source arouse difficulty in material characterization as well as the process design. Other than this results showed that the particle size ≤0.15 mm was suitable for selective recovery of Au, Ag, Fe, Zn, Ni, and Co. However, Cu and Cr can be recovered easily from large size fractions >71 mm of CPCBs. The CPCBs waste of particle size ≤0.15 mm was used in further studies because of the presence of high amount of precious metals.

Table 1. Chemical composition of different particle size fractions of CPCBs

Metals	Concentration (% *w/w*)				
	P1	**P2**	**P3**	**P4**	**P5**
Cu	29.29	4.72	4.62	1.38	7.0
Fe	0.01	0.11	0.21	0.63	2.4
Zn	0.006	0.001	0.01	0.07	0.02
Cr	0.12	0.02	0.11	0.04	0.3
Ni	0.12	0.017	0.09	0.06	0.2
Co	0.001	0.001	0.002	0.002	0.004
Au	0.002	0.001	0.002	0.008	0.03
Ag	0.002	0.006	0.03	0.04	0.14

P1 (≥0.71 mm); P2 (0.35 to 0.71 mm); P3 (0.15 to 0.35 mm); P4 (≤0.15 mm); P5 (≤0.15 mm)

4.2 Effect of digestion time on metallic content of waste CPCBs P4 and P5

Metals content of P4 and P5 of same particle size ≤0.15 mm were analyzed using aqua regia digestion at different interval of time i.e.1, 2 and 3 h. Figure (2) represents metal content with respect to digestion time. The Cu content of P4 and P5 was 1.38%, 2.34%, 2.29% and 7%, 9.8% and 7.85% when digested for 1, 2, and 3 h, respectively. Recovery of precious metal Au in P4 and P5 was maximum (0.008% and 0.03%) when digested for 1 h; increase in leaching time from 2 to 3 h resulted in decreased metal content. The Au content of CPCBs P5 varied from 0.02 to 0.03%, respectively. The Ag content of both P4 and P5 was ranged between 0.04 to 0.05% and 0.1 to 0.14%, respectively. Other metals like Zn, Cr and Ni also showed significant variation in the metals content with respect to digestion time and were given in Fig. (2). In the present study as well as in the literature, it was found that Ag content in the CPCBs was higher than Au. This may be because Au is mainly used for plating and in ICs whereas, Ag is present in plated pins, ICs (integrated circuits), solder junctions with Sn, thin layer on CPCBs and fragments of alloys [26].

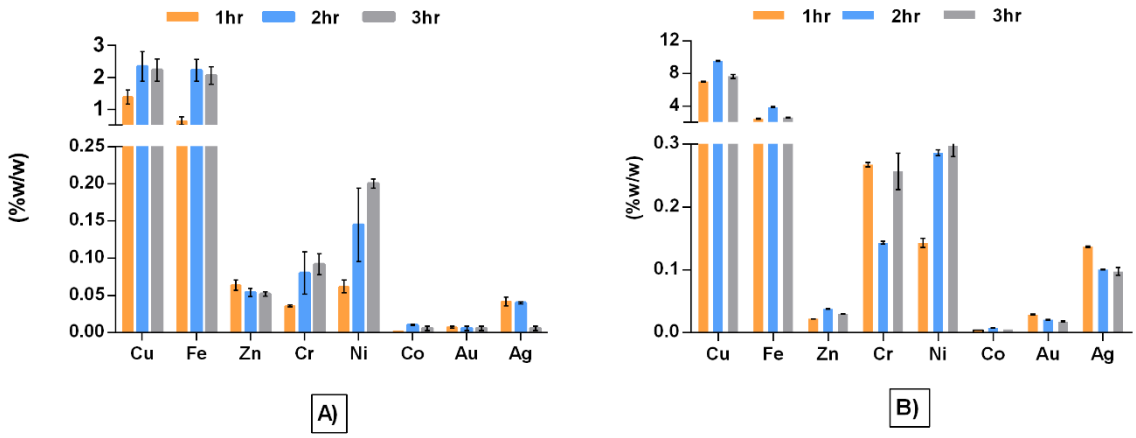

Figure 2: Effect of digestion time on metals content of waste CPCBs: (A) metal content of P4 (particle size ≤ 0.15mm); and (B) metal content of P5

4.3 Isolation, screening and characterization of potent bacterial strains capable of bioleaching of precious metals (Cu, Au, and Ag) from waste CPCBs.

To isolate potent bacteria of interest, site selection is an important factor, as existing site conditions might enrich bacterial population with desired physiological and metabolic machinery. The microorganisms have been reported to play role in biogeochemical cycling of Au and other metals [31]. Therefore, bacterial isolation was achieved using enrichment culture technique from the soil of abandoned gold mine and e-waste refuse of industry, for a period of 30 days. After 30 days, samples were taken from the flask containing 100 g/L pulp density and then serially diluted and spread plated on nutrient agar plates. The results revealed presence of eight different colony morphotypes on nutrient agar plates from abandoned goldmine Solan, HP (Fig. 3). Whereas, single bacterial strain was observed on the agar plate from e-waste refuse of recycling facility, Panipat Haryana. All the nine morphological different colonies were subcultured to obtain pure culture. The mine isolates were Gram +ve whereas, bacterial strain isolated form recycling facility was Gram –ve in nature. The un-inoculated control contained medium only.

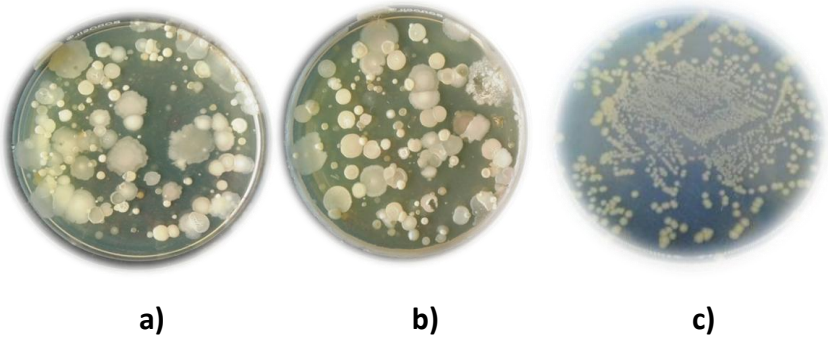

Figure 3: Isolation of bacterial strains from: **a & b)** the abandoned goldmine soil and **c)** e-waste refuse of industry on nutrient agar plates

4.4 Identification using the 16S rRNA gene analysis

Genomic DNA of each bacterial strain was isolated and PCR amplified to obtain 16S rRNA gene sequences and classified according to their similarity with the available 16S rRNA sequences in the GenBank database. The 16S rRNA sequences of each bacterial strain was analyzed using BLASTn search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of NCBI. Based on the highest homology with those of available 16S rRNA sequences, bacterial strains isolated from goldmine were identified as *Bacillus megaterium* SAG1; *Lysinibacillus sphaericus* SAG2; *Bacillus* sp. SAG3; *Bacillus amyloliquefaciens* SAG4; *Bacillus* sp. SAG5; *Brevibacterium frigoritolerans* SAG6; *Chryseomicrobium amylolyticum* SAG7 and *Bacillus safensis* SAG8, respectively. The bacterial strain SAE1 isolated from industrial e-waste refuse was identified as *Pseudomonas balearica* SAE1. The 16S rRNA gene sequence has been submitted to the GenBank database and are available under the accession no: KU163234-KU163241 for SAG1 to SAG8 and KU053282 for SAE1.

4.5 Screening for bioleaching of metals (Cu, Au and Ag) from waste CPCBs

The use of indigenous bacterial strains has been the most practical approach for establishing a bioleaching/bioremediation process because of their natural adaptability to the chemical/pollutants/metals and environmental conditions [32]. Therefore, *C. violaceum* along with nine bacteria viz. *B. megaterium, L. sphaericus, Bacillus* sp. *B. amyloliquefaciens, Bacillus* sp., *B. frigoritolerans, C. amylolyticum, B. safensis* and *P. balearica* were screened for their metal bioleaching potential for seven days. It was observed that all the bacterial isolates had the capability to mobilize metals from ground CPCBs (Fig. 4). *C. violaceum* exhibited maximum bioleach abilities of Au (58.4%) from the PWBs, followed by *P. balearica* SAE1 (56.4%), *B. megaterium* SAG1 (55.26%), *L. sphaericus* SAG2 (46.9%) and *Bacillus* sp. SAG3 (45.3%), respectively (Fig. 4). The amount of Cu bioleached was higher in case of *C. violaceum* (71.6%) followed by *P. balearica* (64.8) and *B. megaterium* (62.6%). The Ag leaching was higher in case of *P. balearica* (26.4%). The metal leaching efficiency of *C. violaceum* is well known and documented [9, 12, 16, 17]. However, the use of *P. balearica,* and *L. sphaericus* in Cu, Au and Ag leaching is reported for the first time. Other than this, control flasks showed 18.22% mobilization of Cu under non growth conditions. Based on precious metals mobilization efficiency, four bacterial strains i.e. *P. balearica, B. megaterium, Bacillus* sp. and *L. sphaericus* along with *C. violaceum* were employed for further optimization to enhance metals recovery from waste CPCBs.

4.5.1 Cyanide Analysis: The efficient bacterial strains were tested for their cyanide leaching potential. It was observed that all the five bacterial strains showed a positive cyanide test which, showed that the biogenic cyanide is responsible for hydrogen cyanide production. Our results are in accordance with previous reports by Natarajan and Ting [33] and Arshadi and Mousavi [24].

4.6 E-waste toxicity assessment and dose-response analysis of potent bacterial strains

The toxicity assessment study are regularly used in toxicology, as a quantitative analysis to show the criteria of maximum treatment concentration (denoted as EC_{100}) and 50% effective concentration (EC_{50}) allowable for metal bioleaching. Therefore, bacterial cells of *B. megaterium, L. sphaericus, Bacillus* sp. and *P. balearica* along with *C. violaceum* were exposed to 10, 50, 100, 150, 200, 300, 400 and 500 g/L of CPCBs pulp density. The growth in terms of CFU/mL was measured for each pulp density along with control. It was observed that *P. balearica* showed 72% inhibition at 500 g/L of e-waste pulp density. Whereas, *B. megaterium, L. sphaericus, Bacillus* sp. and *C. violaceum* showed complete inhibition (100%) at 200-300 g/L of e-waste pulp density. The inhibitory effect of waste CPCBs is due to the presence of heavy metals and other toxic pollutants such as polybrominated diphenyl ethers [34]. To find out EC_{50} values, percent inhibition data was used to generate quantitative dose response curves (Fig. 5). The R^2 values for dose response curves of *P. balearica*, *Bacillus* sp. SAG3, *B. megaterium, L. sphaericus* and *C. violaceum* were 0.97, 0.95, 0.92, 0.88 and 0.91, respectively. The EC_{50} values for *P. balearica*, *Bacillus* sp. SAG3, *B. megaterium* and *L. sphaericus* SAG2 were Log 2.5 (325.7 g/L), 2.1 (128.9 g/L), 1.9 (98.7 g/L) and 1.9 (90.8 g/L), respectively. Whereas, EC_{50} for *C. violaceum* was Log 1.9 (83.70 g/L). The higher

resistance of indigenous bacteria towards CPCBs toxicity may be attributed to their habitat "gold mine/e-waste recycling site", which have developed metabolic machinery to resist the toxic effects. This clearly indicates the technological feasibility and viable operation range for bioleaching of metals from e-waste using native isolates.

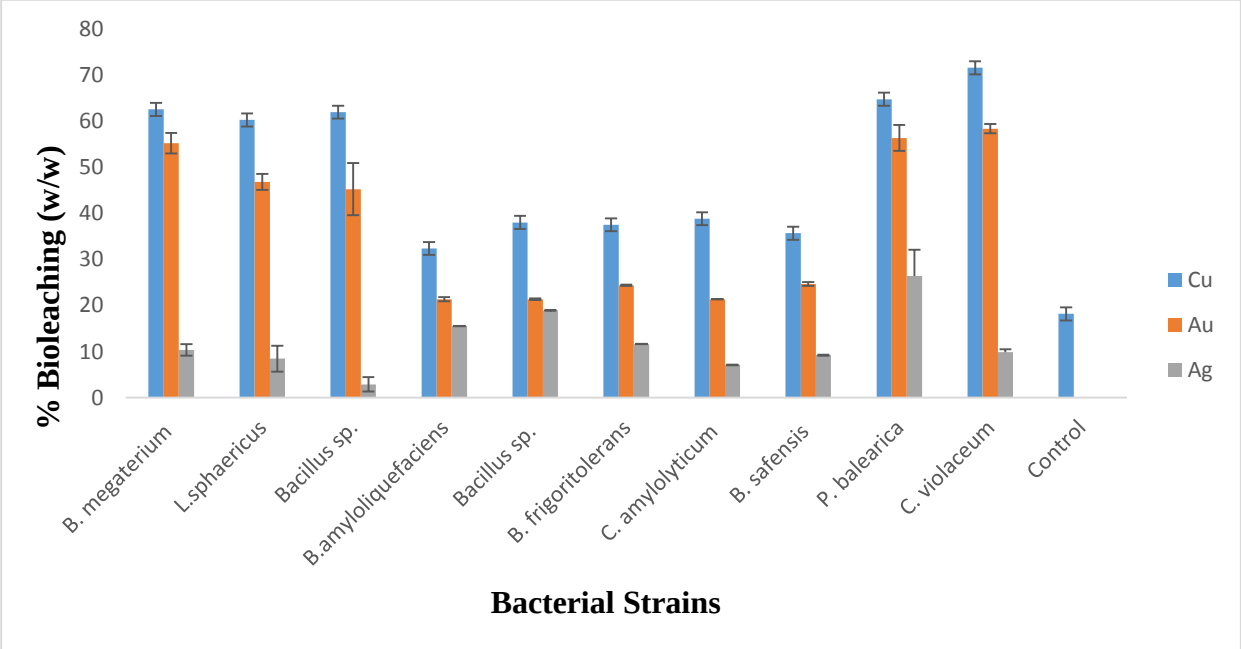

Figure 4: Screening of isolated bacterial strains for bioleaching precious metals (Cu, Au and Ag) from waste CPCBs. The data represented here is the average values obtained from duplicate experiments at significant level ($p < 0.05$) based on two-way ANOVA analysis.

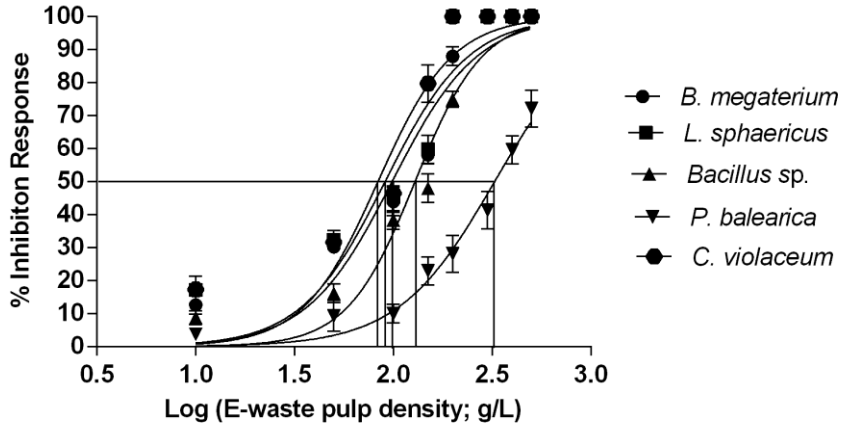

Figure 5: Dose–response curves of e-waste toxicity predicted using equation "*log (agonist) versus normalized response--variable slope*" of GraphPad Prism 6.

4.7 Optimization of process for maximum recovery of metals (Cu, Au and Ag) from waste CPCBs

4.7.1 Effect of Pulp density:- The variation in bioleaching pattern of all five bacterial strain was observed when applied in presence of 10, 50 and 100 g/L of CPCBs concentration. The results of this study revealed maximum metal mobilization and bacterial growth at pulp density of 10 g/L; a further increase of which led to a significant decrease in Cu, Au and Ag mobilization. The low metals mobilization at higher pulp density may be due to toxic effects of the e-waste, which have inhibited the bacterial metabolism [13], thereby resulting in reduced growth and poor lixiviant production. Among all the bacterial strains analyzed, *C. violaceum* showed of higher mobilization of Cu (71.63%) and Au (58.4%) whereas, Ag (26.5%) was higher in case of *P. balearica*. The results are in accordance with Pradhan and Kumar [12], who reported higher mobilization of Cu (79%) and Au (69%) from e-waste at 1% pulp density compared to 5% and 10% pulp density, respectively, using *C. violaceum*. Natarajan and Ting [16], performed bioleaching of pretreated and untreated electronic scrap material (ESM) at pulp densities of 0.5, 1, 2 and 4%, respectively, using *C. violaceum*. Their results showed higher mobilization of Cu and Au at 0.5% pulp density with both untreated and pretreated ESM. Another study by Işıldar et al. [35], reported higher recovery of Au (44%) from WPCBs using *P. putida* at 1% pulp density compared to pulp densities of 2.5 and 5%, respectively. The low metal mobilization at higher pulp densities (50 &100 g/L) was not economically feasible, therefore 10g/L concentration of e-waste would be employed for further experimental purposes.

4.7.2 Effect of Temperature:- To find out the optimum temperature, the two-step bioleaching was performed at temperatures of 25, 30, 35, and 40°C, while keeping all other factors constant. Maximum mobilization of Cu, Au and Ag occurred at 30°C. This corresponds to the fact that temperature variation influenced the degree of secondary metabolite (HCN) formation and maximum cyanide production occurs at a temperature range of 25-30°C [36]. The percentage of Cu, Au and Ag mobilized by different bacteria at 30°C was 71.7%, 58.9% & 7.3% by *C. violaceum*; 67.2%, 56.2%, 26.6% by *P. balearica*; 66.1%, 57.2% & 6.7 by *B. megaterium*; 64.9%, 56.1% & 10.5% by *L. sphaericus*; 61.78%, 50.9% & 3.8% by *Bacillus* sp., respectively. Thus 30°C temperature was considered as optimum; which is in accordance with former bioleaching studies [27, 35, 36]. Additionally, temperature 30°C offers the advantage of low energy requirement during industrial-scale application, thereby potentially reducing the cost of the process.

4.7.3 Effect of glycine concentration: Since hydrocyanic acid is directly produced from glycine, the influence of different glycine concentrations i.e. 2.5, 5.0, 7.5 and 10 g/L on bioleaching of Cu, Au, and Ag from WPCBs was investigated. The increase in glycine concentration from 2.5-5.0 g/L increased the metal mobilization for all bacteria employed for bioleaching. Further, increase in glycine concentration from 5.0 to 10.0 g/L resulted in decreased metal mobilization except for *C. violaceum* and *P. balearica*. At 10 g/L glycine concentration, a decreased metal mobilization was observed. This is due to inhibitory effects of glycine on bacteria at a concentration above 7.5 g/L. The optimum glycine concentration during bioleaching was 5 g/L for *B. megaterium* (Cu- 65.5%,

Au-56.8% & Ag-6.6%), *L. sphaericus* (Cu-64.2%, Au-55.8%, & Ag-10.5%) and *Bacillus* sp. (Cu-63.4%, Au-52.4% & Ag 4.2%). Whereas, *C. violaceum* (Cu- 76.9%, Au- 60.8% & Ag- 6.8%) and *P. balearica* (Cu-69.7%, Au-62.1%, Ag-27.9%) showed maximum mobilization at a glycine concentration of 7.5 g/L. Our results are on the same line by Işıldar et al. [35] and Shin et al. [37] reported inhibitory effects of glycine above 7.5 g/L using *P. fluorescens* and *P. putida* during the bioleaching of metals from e-waste. However, the metal leaching efficiency was statistically not significant when glycine concentration increased from 5.0 to 7.5g/L. Therefore, glycine concentration 5 g/L was taken as optimum for further experiments.

4.7.4 Effect of pH: The pKa of HCN is 9.3, alkaline pH increases the availability of aqueous cyanide ions (CN^-) and thereby enhances the metal mobilization efficiency. However, optimum bacterial growth occurs at physiological pH range of 7 to 8 [16]. An equilibrium reaction of bacterial cyanide production can be represented as per equation (2) [33]:

$$HCN \leftrightarrow H^+ + CN^- \qquad (2)$$

Therefore, metal mobilization ability of bacterial strains were tested at different initial pH (7, 8 and 9) in LB medium. It was observed that maximum metals mobilization takes place at pH 9.0 and least at pH 7.0 except *L. sphaericus*, where maximum metal mobilization was observed at a pH of 8.0. This is because at pH 7 equilibrium shifts to produce more HCN gas, which is volatile and less water-soluble. At alkaline pH 9, the equilibrium shifts toward aqueous cyanide ions (CN^-), making it highly available for metals solubilization/complexation [33]. Among all the bacterial strains average metals mobilization was maximum with *C. violaceum* followed by *P. balearica*, *B. megaterium*, *Bacillus* sp. and *L. sphaericus*. Out of five bacterial strain tested, *C. violaceum* showed higher mobilization of Cu (87.5%) and Au (73.6%) whereas, Ag (33.8%) leaching was maximum by *P. balearica* respectively. Chi et al. [14], performed bioleaching of Cu and Au from waste mobile phone PCBs using *C. violaceum*. They reported that the dissolution of Cu and Au increased from 4.9 to 11.4% and 7.78 to 10.8% in 8 days with the increase in pH from 8-10 and 8-11, respectively. Natarajan and Ting [16], reported the highest recovery Au (22.5%) at pH 9.5 using mutated *C. violaceum*. In another study, they reported 30% and 95.7% of Au and Cu recovery using spent medium leaching at pH 10 [33]. These studies indicate that metal mobilization using cyanogenic bacteria under alkaline conditions can be of industrial interest.

4.8 Validation of optimized parameters

Two-step bioleaching of e-waste was carried for a time period of seven days at pulp density of 1%, glycine concentration of 5 g/L; a temperature of 30ºC; a particle size of ≤0.15 mm and a pH of 9.0 except for *L. sphaericus* where optimized pH was 8.0. Bioleaching profile of Cu, Au and Ag by all bacterial strains was shown in Figure 6. Among all the bacterial strains tested, *C. violaceum* showed maximum mobilization of Cu (87.5±7%) and Au (73.6±4%) (Fig. 6). Whereas, the Ag (33.8±4%) mobilization was higher in case of *P. balearica.* The native bacterial strain *P. balearica* showed highest metal mobilization (Cu-77.4±7%, Au-68.5±5%, Ag- 33.8±4%) followed by *B. megaterium* (Cu-72.7±5%, Au-66.6±6% and Ag-15.3±3%) (Fig. 13). The lowest

mobilization of Cu (64.3%) and Au (57.4%) was attained by *L. Sphaericus* whereas, Ag (8.7%) was lowest mobilized by *Bacillus* sp. The high bioleaching efficiency of bacterial strains is attributed to greater tolerance of microorganisms to metal toxicity, cyanide production, and formation of stable complexes in the presence of e-waste. Similar studies on metals bioleaching from e-waste using cyanogenic microorganisms by different researchers reported differences in metal mobilization efficiency.

Farmarzi et al. [15], obtained a maximum dissolution of 14.9% of Au (dicyanoaurate) from shredded PCBs using *C. violaceum* in a one-step bioleaching (bacterial inoculum and e-waste is added directly to the growth medium). Brandl et al. [11], reported 68.5% and 5% of Au and Ag dissolution from shredded PCBs and silver containing jewelry waste using cyanogenic microorganisms. Pradhan and Kumar [12] reported 83, 73 and 8% recovery of Cu, Au and Ag, respectively, from e-waste using a mixture of *C. violaceum* and *P. aeruginosa*. Another study reported that *P. chlororaphis* leached 52.3, 12.1 and 8.2% of Cu, Au and Ag, respectively, from waste PCBs [36]. Arshadi et al. [24], optimized bioleaching process through response surface methodology to recover metals from mobile phone PCBs using *B. megaterium*. Under optimal conditions they reported 72% of Cu and 65 g Au/ton mobile phone PCBs.

4.9 RSM based optimization

P. balearica showed higher resistance to CPCBs toxicity and higher Ag leaching among all the strains tested. Therefore, to further enhance its metals leaching capability for industrial suitability, the CCD of RSM was employed to optimize different factors (pH, pulp density, temperature, and glycine concentration) effecting recovery of metals during bioleaching. Statistical results of ANOVA suggested a quadratic model for Cu, Au and Ag recovery. The significance of the model was depicted by "model *F*-values" of 29.9 (Cu), 8.03 (Au) and 26.9 (Ag). The values of Prob > F for the models that were less than 0.05 indicated that the models were statistically significant. The ratio of 19.8, 10.4 and 19.3 for model Cu, Au and Ag recovery indicates an adequate signal and can be used to navigate the design space defined by CCD [23]. A lower coefficient of variance (CV) for model Cu (7.1%), Au (15.3%) and Ag (15.6%) recovery indicates that experiments conducted were specific and reliable [38]. According to Amiri et al. [39], the quality of fit of the polynomial model equations (Eqs. 3, 4 and 5) were expressed by the coefficient of determination (R^2). The R^2 values for model Cu, Au and Ag recovery were 0.96, 0.88 and 0.96, whereas adjusted R^2 values were 0.93, 0.77 and 0.92, respectively. The higher R^2 values confirm that models are capable of representing the experimental system [27]. Three-dimensional (3D) response surface plots were used to study the interaction effects of different parameters on Cu, Au and Ag mobilization. The CCD-RSM suggested a quadratic model for recovery of Cu, Au and Ag on the basis of observed responses of 30 experimental runs. The quadratic polynomial equation in terms of coded factors is as follows in equation (3-5):

$$Y_{Cu} = 61.28 + 1.80A - 9.64B - 3.72C + 1.52D - 0.48AB + 0.37AC - 0.17AD + 1.81BC - 2.24BD - 0.64CD - 4.5A^2 - 0.82B^2 - 4.86C^2 - 6.68D^2 \quad (3)$$

$$Y_{Au} = 50.08 + 0.72A - 9.25B - 2.57C + 2.09D - 0.50AB - 0.29AC + 0.09AD + 1.23BC - 0.32BD - 1.10CD - 3.92A^2 - 0.66B^2 - 4.37C^2 - 5.75D^2 \quad (4)$$

$$Y_{Ag} = 23.34 + 1.06A - 6.43B - 1.81C + 0.25D - 1.61AB - 0.67AC - 0.33AD + 2.24BC - 0.93BD - 0.42CD - 2.97A^2 + 0.10B^2 - 2.93C^2 - 4.47D^2 \quad (5)$$

A, *B*, *C*, and *D* represent the initial pH, pulp density (g/L), glycine concentration (g/L), and temperature (°C), where multiple between-two variables show their interactions with each other. The model for Cu ($p < 0.001$), Au ($p < 0.001$) and Ag ($p < 0.001$) recovery was statistically significant at high confidence level.

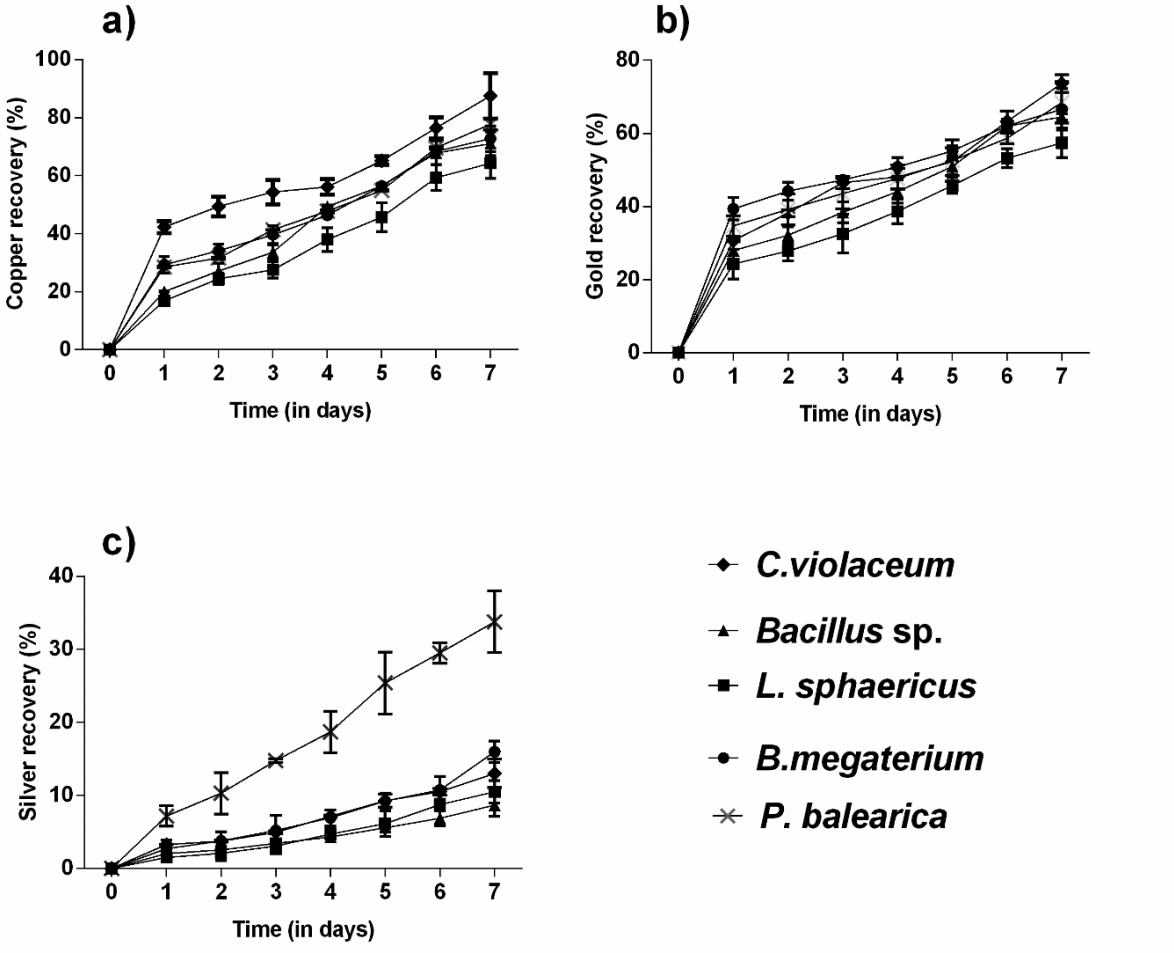

Figure 6: Effect of optimized parameters on metals mobilization (%) during bioleaching of e-waste by different bacterial strains: a) % Cu mobilization; b) % Au mobilization; c) % Ag mobilization. The data presented in this figure presents the mean of values obtained from duplicate experiments.

4.9.1 Model validation

To confirm the validity of models, bioleaching experiments (duplicates) were performed under optimal conditions as predicted by the software. According to the software numerical optimization, the maximum predicted recovery of Cu, Au and Ag should be 80.4%, 67.6% and 39.2%, respectively, (Table 2) at initial pH 8.6, pulp density 5 g/L, temperature 31.2°C, and glycine concentration of 6.8 g/L, respectively. The confirmatory results showed that the obtained responses for Cu (81.7%), Au (73.9%) and Ag (41.6%) were in good agreement with predicted responses, hence validating the accuracy of the models. Control experiments in absence of bacteria showed mobilization of Cu (21.6%) but no Au and Ag was mobilized.

Table 2. Predicted vs. observed responses of Cu, Au & Ag under optimal conditions

Response	Predicted response	Observed response	95% PI low	95% PI high
Cu recovery (%)	80.4	81.7±3.4	71.2	89.6
Au recovery (%)	67.6	73.9±5.9	51.7	83.6
Ag recovery (%)	39.2	41.6±2.4	32.8	45.7

4.10 Bioleaching of Cu, Au and Ag in 1 L medium volume under optimized conditions

C. violaceum, *P. balearica*, and *B. megaterium* were subjected for two-step bioleaching in shake flasks of 2000 mL capacity containing 1000 mL LB medium. Bioleaching was performed under the conditions optimized through OFAT approach i.e. a pulp density of 10 g/L, a glycine concentration of 5 g/L, a temperature of 30 °C, and a pH of 9.0, respectively.

From the result of Figure (7), it was observed that all the three bacterial isolates showed similar metals leaching ability as in case of 100 mL medium volume. *C. violaceum* showed highest metals leaching of Cu (82.2±6%), Au (72.9±5%) and Ag (14.2±3%), respectively, after seven days of bioleaching. *P. balearica* was able to mobilize 73.8±5, 64.6±5 and 31.2±4% of Cu, Au and Ag, respectively. *B. megaterium* mobilized 69.4±7, 65.2±6, 11.8±2% of Cu, Au, and Ag, respectively. Overall, *C. violaceum* showed maximum mobilization of Cu and Au whereas, Ag mobilization was maximum with *P. balearica*.

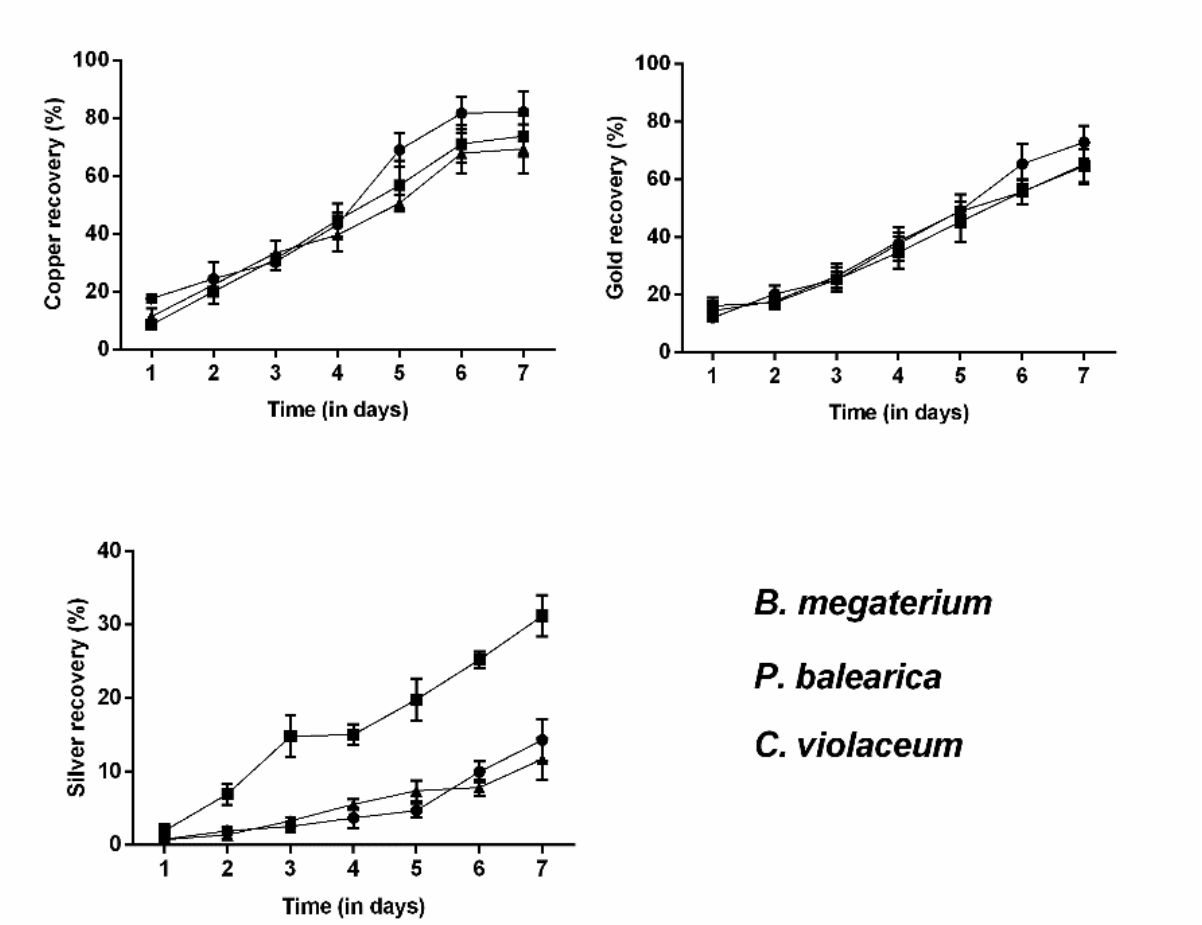

Figure 7: Bioleaching of metals from CPCBs P4 in shake flasks reactors of 2L capacity under optimized conditions.

4.11 Pretreatment of waste CPCBs-P5

The CPCBs waste P5 contains high amount of Cu and base metals. In the literature, it was reported that Cu and other base metals forms complexes with cyanide and their presence at higher concentration in e-waste causes hindrance in recovery of gold [9, 16]. Thus, CPCBs P5 was pretreated with nitric acid (HNO_3) to remove and recover Cu and other base metals in order to enhance Au recovery. The pretreatment with acid lead to 82.1, 57.6, 70.4, 65.8, 47.4 and 90.2% of Cu, Ni, Fe, Cr, Ag and Zn recovery without significantly altering the concentration of Au (Table 3). Nitric acid mediated dissolution of Cu is presented in equation (6). The results corroborated with the study of Sahni et al. [9] and Natarajan and Ting [16], reported nearly 72% and 80% removal of copper.

$$3Cu + 8HNO_3 \rightarrow 3Cu^{2+} + 6NO_3^- + 2NO\uparrow + 4H_2O \qquad (6)$$

Table 3: Chemical composition of pretreated and untreated waste P5 using acid digestion by aqua regia

Metals	Untreated (% w/w)	Pretreated (% w/w)	% Removal with pretreatment
Cu	9.8 ± 0.42	1.70±0.14	82.6
Fe	4.01 ± 0.22	1.6±0.08	60.1
Ni	0.11 ± 0.08	0.004±0.004	96.36
Cr	0.15 ± 0.001	0.1±0.008	33.33
Co	0.008 ± 0.09	0.004±0.008	50.00
Zn	0.04± 0.015	0.004±0.001	90.00
Ag	0.14 ± 0.009	0.07±0.001	47.36
Au	0.03 ± 0.002	0.025±0.004	15.44

4.12 Comparative bioleaching of untreated and pretreated waste P5

Since, all the five bacterial isolates exhibited good Cu, Au and Ag bioleaching efficiency from waste CPCBs dust. However, to confirm industrial suitability of these bacterial isolates, it is necessary to investigate their bioleaching potential from metals enriched waste. Out of total five, three bacterial strain viz. *C. violaceum*, *P. balearica* and *B. megaterium* found efficient during bioleaching from CPCBs waste P4 (dust); were employed for bioleaching from waste P5. From the results of Figure (8), it was observed that *C. violaceum* (48.4%) showed maximum leaching of Cu followed by *P. balearica* (44.3%) and *B. megaterium* (36.5%), respectively. Whereas, Au and Ag leaching was significantly low. This may be due to high concentration (9.8%) of copper present in the waste P5, which might have consumed all the lixiviant produced by bacteria resulted in poor metal mobilization Au and Ag. *C. violaceum, P. balearica* and *B. megaterium* were employed for bioleaching of Cu, Au and Ag from pretreated waste P5 to enhance the Au solubilization under previously optimized conditions. Thus waste P5 was treated using HNO_3 to remove Cu and other base metals in order to enhance gold dissolution. Bioleaching of pretreated waste P5 using cyanogenic bacteria resulted in 81.2, 79.9, and 69.8% mobilization of Cu by *C. violaceum, P. balearica* and *B. megaterium*, respectively. The Au recovery from pretreated waste P5 was enhanced up to 52.4%, 47% and 42% compared to 7.1%, 4.6%, 4.2% bioleaching from virgin waste P5 by *C. violaceum*, *P. balearica*, and *B. megaterium*, respectively. In case of Ag bioleaching from pretreated e-waste bacterial strains *C. violaceum*, *P. balearica*, and *B. megaterium* showed 18.3, 25.9 and 16.3% mobilization of Ag. Un-inoculated control showed mobilization of Cu in both untreated (22.3±5%) and pretreated (31.9±5%) e-waste, but no Au and Ag was detected during the entire bioleaching period. The results showed that bioleaching using cyanogenic microorganisms from metals enriched waste CPCBs is not economic. However, a combination of chemical and biological methods may be successful in recovery of metals from e-waste.

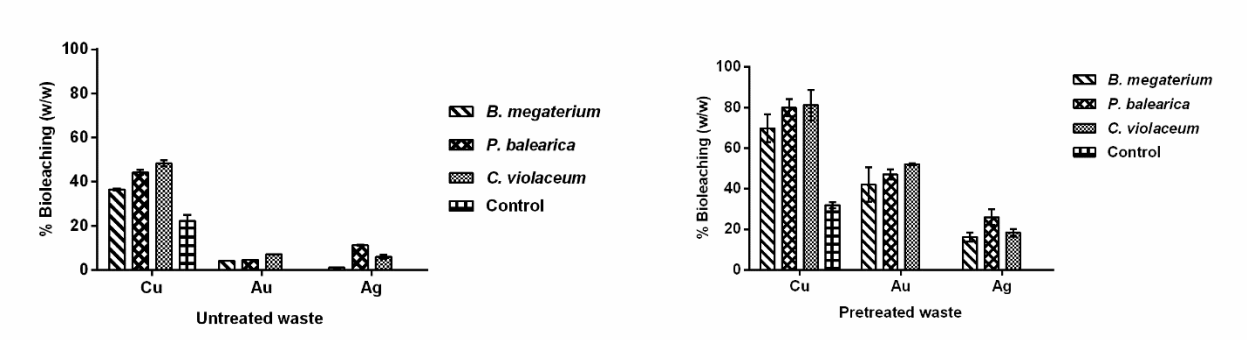

Figure 8: Comparative bioleaching of Cu, Au and Ag from untreated and pretreated waste P5

4.13 Final Disposal of the e-waste

The residual CPCBs P4 and P5 after bioleaching were characterized for metal content using aqua regia digestion method. The metallic composition of both the residual wastes were presented in Table 4. The Cu content in residual waste P4 was 0.15% compared to the initial 2.34%. The Fe (0.56%) content was maximum among all the metals analyzed. This may be because Cu has high affinity towards cyanide, which form water soluble complex during cyanogenic bioleaching. The precious metals Au and Ag in residual waste P4 were 0.002 and 0.03% compared to the initial 0.008 and 0.04%, respectively. Other metals like Ni, Co, Zn and Cr were 0.004, 0.001, 0.009, and 0.07%, respectively, were also present but in significantly low quantities. Although bioleaching using cyanogenic microorganisms have recovered metals from waste CPCBs yet complete recovery of metals were not observed. Similarly in the residual waste P5, Fe (0.65%) content was maximum than Cu (0.5%). The total Cu and Fe present in the P5 was 9.8% and 4.0%. The metals from P5 were leached in significant amount through a combination of chemical (pretreatment using HNO_3) and biological methods (bioleaching using cyanogenic bacteria). Therefore, the final waste need special attention and safe disposal. In our case we have collected the residual of bioleaching and chemical leaching and handed over to our industrial collaborator Exigo recycling Pvt. Ltd. Where they will safely dispose it off at their treatment storage and disposal facility (TSDF). The final disposal of e-waste after bioleaching of metals is still challenging.

Table 4: Characterization of metals content in residual (after bioleaching) e-waste

Metals	**Concentration (% *w/w*)**	
	P4	**P5**
Cu	0.15 ± 0.02	0.5± 0.01
Fe	0.56 ± 0.06	0.65±0.1
Ni	0.004± 0.006	0.08±0.02
Co	0.001 ± 0.01	0.001±0.001
Cr	0.07 ± 0.005	0.08±0.02
Zn	0.009 ± 0.001	0.002±0.002
Ag	0.03 ± 0.005	0.05±0.009
Au	0.002 ± 0.007	0.01±0.002

5. CONCLUSION

The present study have addressed the problem of e-waste treatment and its safe disposal; an important global issue. In the study, e-waste of CPCBs was treated to recover precious metals (Cu, Au and Ag) using biological and chemo-biological methods. Bacterial strains indigenous to abandoned gold mine an e-waste recycling facility were explored and employed for bioleaching of waste CPCBs. Initial screening for bioleaching of Cu, Au and Ag from waste CPCBs showed four prominent bacterial strains viz. *P. balearica, B. megaterium*, *Bacillus* sp. and *L. sphaericus* along with *C. violaceum*. The higher resistance of indigenous bacterial strains towards e-waste toxicity confirms the viable operation range and technological feasibility of metals bioleaching from waste CPCBs. Process optimization was carried to enhance metals leaching potential of bacterial strains using OFAT and RSM based approaches. Under optimized conditions, *C. violaceum* showed highest mobilization of Cu (87.5%) and Au (73.6%), respectively, from waste CPCBs dust. Whereas, Ag (33.8%) mobilization was maximum for *P. balearica*. It is clear from the research, that bioleaching using alkaline cyanogenic microorganisms is promising, when used to treat CPCBs of low metallic concentration for example waste CPCBs dust. However, in case of virgin CPCBs containing higher concentration of metals, a combination of chemical and biological method is more effective. Through our chemo-biohybrid approach; approximately 95%, 60%, and 64% of Cu, Au and Ag were mobilized from virgin waste CPCBs. Further, residual CPCBs of bioleaching were characterized for its metals content and showed presence of metals ions. Therefore, the residual waste was sent back to the authorized recyclers i.e. Exigo Recycling Pvt. Ltd. for final disposal through TSDF.

6. FUTURE PROSPECTS

The microorganisms used in the bioleaching are limited to *Acidithiobacillus thiooxidans, Acidthiobacillus ferrooxidans, C. violaceum, B. megaterium* and *Pseudomonas sp.* Thus there is a great scope to explore for novel microorganism, innovation interventions so as to meet profitable metals recovery. Long leaching time and metals recovery at low pulp density are the barriers in the industrial success of bioleaching from e-waste. This can be overcome by genetic manipulations of the microbes. In the present work the novel bacterial strain *P. balearica* isolated from indigenous e-waste recycling site with higher e-waste toxicity tolerance (EC_{50}=325.7 g/L) have potential to be carried forward for research in order to overcome the long leaching time and low pulp density using genetic engineering techniques. To enhance industrial acceptability, bioleaching potential of isolated indigenous bacterial strains need to be tested for all type of e-waste; for example, mobile phone PCBs and hard disk drive boards etc. Thus, this field (e-waste bioleaching) requires adaptation of hybrid techniques which includes integration of microbiology, biotechnology, engineering and metallurgy. In addition, a comparative transcriptomics, proteomics and/or metabolomics analysis may be conducted between native (isolated) strains from present study and standard strains from ATCC to convey their promising metals bioleaching potential. Research can also be focused on selectivity of the target metallic ions against the strains tested.

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1. **Kumar A**, Saini H S, Kumar S. 2018. Enhancement of gold and silver recovery from discarded computer printed circuit boards by *Pseudomonas balearica* SAE1 using response surface methodology (RSM). *3Biotech*; https://doi.org/10.1007/s13205-018-1129-y. **(I.F 1.36; SCOPUS, UGC Journal list No. 99)**
2. **Kumar A**, Saini H S, Kumar S. 2018. Bioleaching of gold and silver from waste printed circuit boards by *Pseudomonas balearica* SAE1. *Current Microbiology*; 75(2): 194-201. **(I.F 1.32; SCOPUS, UGC Journal list No. 14256)**
3. Sahni A, **Kumar A** and Kumar S. 2016. Chemo-biohydrometallurgy—A hybrid technology to recover metals from obsolete mobile SIM cards. *Environmental Nanotechnology, Monitoring & Management*; 6: 130-133. **(SCOPUS, UGC Journal list No. 19809)**
4. **Kumar A**, Saini H S, Şengör S, Sani R K, Kumar S. Bioleaching of metals from waste printed circuit boards using abandoned gold mine isolates. (Under preparation).

CONFERENCES/WORKSHOPS

1. **Kumar A,** Saini H S and Kumar S. Bioleaching of e-waste for precious recovery using cyanogenic bacteria. In International Conference on Innovative Research in Engineering, Science & Technology at Eternal University, Baru Sahib, Himachal Pradesh (7-8 April, 2017) -[Oral Presentation by Kumar A].
2. **Kumar A,** Patial G, Saini H S and Kumar S. Development of sustainable bioleaching technology for precious metals recovery from e-waste. In a National Seminar on issues of E-waste Management" at Govt. Degree College, Arki, Solan, Himachal Pradesh (17 December 2016) – [Oral Presentation by Kumar A].
3. **Kumar A,** Dhammi P, Saini H S and Kumar S. Development of integrated treatment systems for resource recovery and bioremediation of e-waste. National Workshop on "Recent trends in environmental sciences and carbon management" organized by Central University of Himachal Pradesh, Dharamshala, H.P. (19-20 Nov. 2015) **(Best Presentation award**-Poster presentation by Kumar A).
4. **Kumar A**, Sharda M, Dhammi P, Saini H S and Kumar S. Development of sustainable bioleaching technology for recovery of precious metals (Au, Ag and Pt.) from e-waste and biodegradation of polybrominated aromatic fire retardants**.** 1st Himachal Pradesh science congress on Role of Science and Technology for Sustainable Development in H.P (**Best Oral Presentation Award**-Oral Presentation by Kumar S).
5. **Kumar A,** Saini H S and Kumar S. Exploration of novel bacterial species from the abandoned gold mine for bioleaching and enhancement of gold recovery from e-waste. International Conference on Life Sciences, Informatics, Food and Environment, August 29-30, 2014 at JIIT, Noida (**Best Poster Presentation Award**- Poster presentation by Kumar A).

NCBI GENBANK SUBMISSION

Kumar A and Kumar S (2015)
Accession No.:- KU053282, KU163234, KU163235, KU163236, KU163237, KU163238, KU163239, KU163240, KU163241.

ACKNOWLWDGEMENT

I am grateful to Department of Biotechnology, Govt. of India for providing financial support (BT/PR7478/BCE/8/951/2013). I am also grateful to Exigo Recycling Pvt. Ltd. for gifting us e-waste. The Jaypee University of Information Technology, is also acknowledged for providing necessary facilities to carry out the research work.

Signature of the Student **Signature of the Supervisor**